PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

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PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Rule 71.1)

Date of mailing *(day/month/year)*	13.04.2004
Applicant's or agent's file reference: SJW/9179 WO	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB 03/00959	06.03.2003	09.03.2002

Applicant
THE UNIVERSITY OF NOTTINGHAM

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

The applicant's attention is drawn to Article 33(5), which provides that the criteria of novelty, inventive step and industrial applicability described in Article 33(2) to (4) merely serve the purposes of international preliminary examination and that "any Contracting State may apply additional or different criteria for the purposes of deciding whether, in that State, the claimed inventions is patentable or not" (see also Article 27(5)). Such additional criteria may relate, for example, to exemptions from patentability, requirements for enabling disclosure, clarity and support for the claims.

Name and mailing address of the international preliminary examining authority:

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PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference SJW/9179 WO	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/GB 03/00959	International filing date *(day/month/year)* 06.03.2003	Priority date *(day/month/year)* 09.03.2002
International Patent Classification (IPC) or both national classification and IPC A61K35/64		
Applicant THE UNIVERSITY OF NOTTINGHAM		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 11 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 3 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
03.10.2003	13.04.2004
Name and mailing address of the international preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized Officer Gonzalez Ramon, N Telephone No. +31 70 340-3466

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB 03/00959

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-11 as originally filed

Claims, Numbers

1-17 received on 18.03.2004 with letter of 17.03.2004

Drawings, Sheets

1/10-10/10 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☒ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

   see separate sheet

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application,

   ☒ claims Nos. 1-17 partially

   because:

   ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (specify):

   ☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☒ no international search report has been established for the said claims Nos. 1-17 partially

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the Standard.

   ☐ the computer readable form has not been furnished or does not comply with the Standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	
	No:	Claims	1, 4-13, 16, 17
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-17
Industrial applicability (IA)	Yes:	Claims	
	No:	Claims	see separate sheet

2. Citations and explanations

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB 03/00959

see separate sheet

Amendments (Rule 70(2)(c) PCT

The international preliminary authority considers that the amendments filed 18.03.2004 go beyond the disclosure of the international application as filed and the report has been established as if such amendment has not been made.

The reasons therefore are the following:
The subject matter of amended claims 10-12 reading "a composition comprising as an active component a substance.. **isolated from... for the degradation of biofilms**".
Said amendments consist on an unallowable generalisation of subject matter present on examples 3 (for claim 12) and example 4 (for claims 10 and 11).
Said examples fail to disclose a composition comprising as an active component "a substance... **isolated from**..." as claimed, but merely determine the presence of lactonase, glycosidase activity in extracts of L. sericata of undefined composition.
Moreover basis for said generalisation of the use for the general purpose of "degradation of biofilms" is also not present in the application document as filed where only activity against P aeruginosa is described

Re Item III
Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

In the present application, the International Searching Authority has restricted the search under the following objections under Articles 5 and 6 PCT.

Present claims 1-17 relate to compounds defined by reference to pharmacological characteristics or properties, namely: "antibiotic" (claims 6, 8, 14).
The claims cover all compounds having these characteristics or properties, whereas the

application provides support within the meaning of Article 6 PCT and disclosure within the meaning of Article 5 PCT for only a very limited number of such compounds.

Furthermore present claims 1-17 relate to compounds defined by reference to pharmacological characteristics or properties: "substance having N-acyl homoserine lactone degradant activity" (claims 1, 10), "antibiotic" (claims 6, 8, 14); " a serine proteinase" (claim 11); "a glycosidase" (claim 12); "a substance having cecropin-like activity" (claim 13).
No support is to be found in the present application for the substances with "N-acyl homoserine lactone degradant activity, serine proteinase, glycosidase, cecropin-like activity" as claimed.

No particular isolated substance from secretions of Lucilia sericata is effectively disclosed throughout the present application: all the examples presented refer to the activity of whole crude excretions/secretions (ES) of the Lucilia sericata larvae where no particular substance is isolated. Neither the determination of the activity of a substance obtained from excretions/secretions of Lucilia sericata as claimed is effectively described.
Consequently the subject matter of present claims 1-17 does not fulfill the requirements of Article 5 PCT.

Support is only to be found in the present application for those parts relating to the compounds encompassed by the examples and those specifically defined by chemical name in claims 7, 9, 15.

Furthermore claims 10-13, 16 relate to an extremely large number of possible compounds vaguely defined as "analogues" (claims 10-13) or "synthetic analogue" (claim 16). The term "analogue" is vague and unclear and leaves the reader in doubt as to the meaning of the technical feature to which it refers, thereby rendering the definition of the subject-matter of said claims unclear (Article 6 PCT).

Moreover claims 1-17 do not meet the requirements of Article 6 PCT in that the matter for which protection is sought is not defined. The claims attempt to define the subject-matter in terms of the result to be achieved: "substance having N-acyl homoserine lactone degradant activity" (claims 1, 10), "antibiotic" (claims 6, 8, 14); " a serine proteinase" (claim 11); "a glycosidase" (claim 12); "a substance having cecropin-like

activity" (claim 13). In this instance, however, such a formulation is not allowable because it appears possible to define the subject-matter in more concrete terms, viz. in terms of how the effect is to be achieved.

The International Preliminary Examination Authority fully agrees with these objections, therefore no international Preliminary Examination will be carried out in respect of subject-matter which is not covered by the search report (Rule 66.1(e) PCT).

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statements

For the assessment of the present claims 1, 4-7 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

The following documents (D) are referred to in this communication:

D1: EP-A-1020197
D2: WO-A-0131033
D3: J Bacteriol (1935), vol. 30, pp. 253-267
D4: Febs Letters (1998), vol. 425, pp. 131-133
D5: Friedman, E. et al, Third International conference on biotherapy. Jerusalem, May 1998. Abstract.
D6: Maseritz, I. H., Arch. Surg. (1934), vol. 28, pp. 589-607
D7: J. Tissue Viability (1999), 9(4), 127-132.

The document (D) was not cited in the international search report. A copy of the document is appended hereto.

D8: Friis-Moller A. et al, 11th Conference of the European Wound Management Association. Dublin, 17-19th May 2001. Poster 34.

Novelty (Art 33(2) PCT).

The subject-matter of claims 1, 4-13, 16, 17 is not new in the sense of Article 33(2) PCT for the following reasons:

The mechanism of action of a substance as defined "N-acyl homoserine lactone degradant activity, serine proteinase, glycosidase, cecropin-like activity" cannot be considered in itself as a therapeutic application.

The discovery that a substance acts via a biochemical mechanism or route (as i. e. "N-acyl homoserine lactone degradant activity", "serine proteinase", "glycosidase", "cecropin-like activity"), even if representing an important piece of scientific knowledge, still needs to find a practical application in the form of a defined, real treatment of any pathological condition in order to make a technical contribution to the art and to be considered as an invention eligible for patent protection.

When a claim is directed to a therapeutic application of a medicament and the condition to be treated is defined in functional terms, e.g. any condition susceptible of being improved or prevented by "N-acyl homoserine lactone degradant activity, serine proteinase, glycosidase, cecropin-like activity" the claim can be regarded as clear only if instructions, in the form of experimental tests or any testable criteria, are available from the patent documents or from the common general knowledge allowing the skilled person to recognise which conditions fall within the functional definition and accordingly within the scope of the claim.

Present application presents the effective **antimicrobial** activity of secretions/excretions of Lucilia sericata.
The disclosure of the application document as filed refers extensively to said **antimicrobial** activity as well as it is claimed per se (present claims 13, 16).

However, no particular isolated substance from said secretions is isolated neither further analysed/tested to determine the activities claimed.
Consequently, prior art documents disclosing the use of a composition comprising secretions/excretions of Lucilia sericata for the treatment of surface populated by **bacteria capable of producing a biofilm** are to be considered as novelty destroying documents for the subject matter of the present application regardless of the

mechanism of action proposed.

D1 discloses the use of extracts of fly larvae Lucilia sericata for the treatment of chronic or acute wounds. Wound dressing comprising said extracts are also described (see abstract; claims 1, 2).
Consequently the subject matter of claims 1, 4, 10-13, 17 is not novel over D1.

D2 discloses protein secretions of the larvae of Lucilia sericata with serin proteinase, alfa amylase activities for the treatment of wounds. Wound dressing comprising the same are also described (see page 5; claims 6, 13). Therefore the subject matter of claims 11-13, 17 is not novel over D2.

D3 discloses a bactericidal principle in excretions of surgical maggots of Lucilia sericata which destroys etiological agents of pyogenic infections (including Staphylococcus aureus) and its use in the disinfection of wounds (see page 257; discussion). Said bacteria is encompassed under the bacteria capable of forming a biofilm (according to description page 3, lines 7-8).
Consequently the subject matter of claims 1, 4, 5, 10-13 is not novel in view of D3.

D7 discloses the antimicrobial activity of maggot secretions of Lucilia sericata to combat clinical infections in variety of wound types including those caused by antibiotic resistant strains of bacteria: Staphylococcus aureus or Pseudomonas aeuruginosa (see page 128; figures 2-4, 7). Said bacteria are encompassed under the bacteria capable of forming a biofilm (according to description page 3, lines 7-8).
Consequently the subject matter of claims 1, 4, 5, 10-13 is not novel in view of D7.

D8 discloses the antibacterial activity of Lucilia sericata to combat clinical infections in wounds including those caused by antibiotic resistant strains of bacteria: Staphylococcus aureus or Pseudomonas aeruginosa. Said bacteria are encompassed under the bacteria capable of forming a biofilm (according to description page 3, lines 7-8).
The combination of the larvae with antibiotics as ampicillin, ciprofloxacin and erythromycin is also described as synergistic treatment of infected ulcers (see abstract, conclusion).
Consequently the subject matter of claims 1, 4-9, 16 is not novel in view of D8.

Inventive step (Art 33 (3) PCT)

The subject-matter of claims 1-5, 10-13, 16 as far as novel does not involve an inventive step in the sense of Article 33(3) PCT for the following reasons:

According to the applicant (page 1, paragraph 2) the problem underlying the present application is the treatment of surfaces populated by bacteria capable of forming biofilms, as they constitute stable formations resistant to conventional medicaments.

As solution to the problem a composition comprising substances having N-acyl homoserine lactone degradant activity, serine proteinase, glycosidase, cecropin-like activity obtained from secretions/excretions of Lucilia sericata optionally in combination with an antibiotic (tetracycline) is proposed.

D7, which can be considered the closest prior art, already addresses the treatment of surfaces populated by bacteria (including antibiotic resistant strains Staphylococcus aureus and Pseudomonas), in particular wound surfaces with a composition comprising secretions of Lucilia sericata (see abstract, conclusions).

Same bactericidal principle in excretions of the maggots of Lucilia sericata was in fact known on earlier times as described in D3 (see page 254). At the time point said substances named "maggot excretions" for lack of a more specific name.

The difference between D7 and the present application is the fact that a particular substance with a specific mechanism of action (namely N-acyl homoserine lactone degradant activity, serine proteinase, glycosidase, cecropin-like activity) is not explicitly disclosed by D7.

The skilled man, in view of the antimicrobial effect of Lucila sericata secretions shown in D7, would have tried, with the expectation of success, the isolation of said active substance furthermore taking into account the indications for the search of the nature of said "active principle" suggested by D3 (see pages 263-264).

The skilled person in search of the antibacterial active substance and the further indication that the antibacterial agent from maggots is a protein or a peptide (see D5, paragraph 2) would have applied, with the expectation of success, the method for

isolation of an antibacterial protein with specific cecropin-type activity from Sarcophaga peregrina (flesh fly) disclosed in D4 (see page 131, col. 1, table 1) to an extract of analogue fly species as the Lucilia sericata (greenbottle fly) in order to obtain a substance with the claimed antibacterial activity against Staphylococcus aureus, only by application of a routine laboratory protein isolation method and not relying on an inventive skill.

Furthermore the attention of the applicant is also drawn to the fact that all embodiments covered by the claims should satisfy the criteria of inventive step. When the inventive step is solely based on the achievement of a technical effect, such as "homoserine lactone degradant activity, serine proteinase, glycosidase, cecropin-like activity" in the present case, substantially all embodiments (i.e. substance obtained from the secretions/excretions of Lucilia sericata) should exhibit this effect.

However, it is evident that the number of substances is such that it is unlikely that all of them posses the effects claimed.

Moreover taking into account the passage on description page 6 where it is stated that "Lucila sericata secretions showed a slight enhancement of P aeruginosa growth", and it is concluded that "L sericata secretions alone did not have an antibiotic effect on bacterial growth, however there was a synergistic effect with the conventional antibiotic tetracycline" and the further indications that "emulsions of maggots are not bactericidal in action, but on the contrary favour bacterial growth" (see D7, page 599, table 6)

Therefore, as part of the subject matter of claims 1-5, 10-13, 16 does not exhibit this particular technical effect in a credible manner, said subject matter cannot involve inventive step (see Decision of the Board of Appeal T939/92)

Consequently the subject matter of claims 1-5, 10-13, 16 cannot be considered as involving an inventive step.

The particular formulations disclosed in claims 6-9, 14, 15, 17 consisting of a combination of the substance obtained from excretions/secretions of Lucilia sericata with an antibiotic have been suggested by prior art document D8 (see conclusion), and consequently the subject matter of said claims cannot either be considered as inventive.

CLAIMS

1. A method of treating a surface populated by a bacteria capable of producing a biofilm which comprises contacting the surface with a substance having N-acyl homoserine lactone degradant activity obtained from the secretions/excretions of *Lucilia sericata*.

2. A method according to claim 1, wherein the surface is selected from metal surfaces, glass surfaces and the surfaces of plastics materials.

3. A method according to claim 1 or claim 2, wherein the surface is the surface of a medical device or implant.

4. A method according to claim 1, wherein the surface is a wound surface.

5. A method according to any one of claims 1 to 4, wherein the bacteria capable of producing a biofilm is *Pseudomonas aeruginosa* or *Staphylococcus aureus*.

6. A method according to any one of claims 1 to 5, wherein the substance is provided in a composition which additionally comprises one or more antibiotic compound.

7. A method according to claim 6, wherein the antibiotic compound is tetracycline.

8. An antimicrobial composition comprising secretions/excretions isolated from *Lucilia sericata* or analogues thereof and one or more antibiotic compound.

9. A composition according to claim 8, wherein the antibiotic compound is tetracycline.

10. A composition comprising, as an active component, a substance having N-acyl homoserine lactone-degradant activity isolated from secretions/excretions obtained from *Lucilia sericata* or analogues thereof together with a carrier or vehicle, for the degradation of biofilms.

11. A composition comprising, as an active component, a serine proteinase isolated from secretion/excretions obtained from *Lucilia sericata* or analogues thereof together with a carrier or vehicle, for the degradation of biofilms.

12. A composition comprising, as an active component, a glycosidase isolated from secretions/excretions obtained from *Lucilia sericata* or analogues thereof together with a carrier or vehicle, for the degradation of biofilms.

13. An antimicrobial composition comprising, as an active component, a substance having cecropin-like activity isolated from secretions/excretions obtained from *Lucilia sericata* or analogues thereof together with a carrier or vehicle.

14. A composition according to any one of claims 10 to 13 which additionally comprises one or more antibiotic compound.

15. A composition according to claim 14, wherein the antibiotic compound is tetracycline.

16. An antimicrobial composition comprising cell-free haemolymph obtained from *Lucilia sericata* larvae grown in the presence of *Pseudomonas aeruginosa*, or one or more active constituent of said haemolymph or a synthetic analogue of such constituent.

17. A wound dressing comprising a composition according to any one of claims 8 to 16 together with a carrier.